

Nicotinic Receptors and Cholinergic Neurotransmission in the Central Nervous System

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Classic studies of the neuromuscular junction and autonomic ganglia have provided us with a clear picture of nicotinic cholinergic transmission in these tissues. Within the central nervous system (CNS), the elegant experiments of Curtis, Eccles, and associates revealed that nicotinic cholinergic transmission occurs in the ventral horn of the spinal cord, between motoneurons and Renshaw cells. However, as discussed below, evidence for nicotinic cholinergic transmission at other sites in mammalian spinal cord and brain is remarkably fragmentary. We may have been too influenced by earlier studies, assuming too readily that all nAChRs are cholinergic (activated by physiologically released ACh) as well as being cholinceptive.

The existence and prevalence of CNS nicotinic cholinergic transmission is germane to several important issues. First, we should be aware that studying the actions of nicotine in isolation may not tell us as much as we would like about sites of nicotinic cholinergic transmission. Second, with increasing interest in the use of nicotinic antagonists to combat tobacco smoking, it becomes important to identify possible consequences of blocking endogenous nicotinic cholinergic tone. Third, there is a growing expectation that nicotinic cholinergic transmission may be impaired in Alzheimer's disease. This view depends greatly on recent reports that nicotine can alleviate certain cognitive deficits in afflicted individuals^{1,2} and that the nicotinic antagonist mecamylamine can impair cognitive performance in normal human subjects.³ Before central cholinergic systems can be implicated definitively in these drug effects, it is important to know more about possible sites of nicotinic cholinergic transmission and the extent to which such systems are tonically active. This paper serves as a brief summary of current knowledge. Throughout, the abbreviation "nAChRs" refers to nicotinic cholinceptors, whether or not they are activated by endogenous ACh.

NEUROANATOMICAL MAPPING OF CHOLINERGIC SYSTEMS AND NICOTINIC RECEPTORS

The major cholinergic cell groups have been extensively mapped at the light microscopic level in rats, principally by choline acetyltransferase immunocytochemistry combined with retrograde tracing.⁴ Mapping in higher mammals has provided much less detailed hodological information, but the available evidence suggests that the main cholinergic pathways are conserved during evolution, even in humans.^{5,6} Light microscopic studies have revealed the location of cholinergic cell bodies and terminal fields (the latter shown by varicosities), and synaptic formations have been described in a number of regions examined by electron microscopy.

Brain nicotinic receptors have been mapped at low resolution using autoradiography. Several radioligands have been used, revealing two prominent populations. One population is preferentially labeled with high affinity (nanomolar K_d) by tritiated agonists such as [^3H]nicotine, [^3H]ACh, and [^3H]cytisine, whereas the other is selectively labeled by the elapid snake toxin [^{125}I]- α -bungarotoxin.⁷⁻⁹ These two receptor populations are quite differently distributed in brain, and can also be distinguished by affinity isolation, immunological characterization, and molecular genetic approaches.^{10,11} Two other populations of putative nAChR have been identified, characterized by high affinity for the antagonists [^3H]dihydro- β -erythroidine¹² and [^{125}I]neuronal bungarotoxin,¹³ respectively. The latter population appears to represent a distinct receptor subtype, but is present only in low density, at least in rat brain.

Much less is known about the ultrastructural location of nAChRs. The first studies addressing this issue used labeled α -bungarotoxin¹⁴⁻¹⁶ and provided evidence for a (post)synaptic location. However, these pioneering studies should probably be viewed with caution, particularly because statistical analysis was not performed that might have more clearly demonstrated a preferential synaptic location. Another reason for caution is that on mammalian skeletal muscle fibers, α -bungarotoxin binding sites are overwhelmingly *extrasynaptic*, and at autonomic ganglia, predominantly synaptic or *extrasynaptic* locations have both been reported, possibly depending on the species and/or ganglion in question.¹⁷⁻²⁰ Statistical analysis of [^{125}I]- α -bungarotoxin binding in an area of rat hypothalamus nevertheless appeared to favor a synaptic location.²¹

More recently, several groups have raised antibodies to nAChRs or related peptide fragments and reported ultrastructural observations in rat brain. Schröder and colleagues have localized putative nAChRs using a monoclonal antibody (WF6) originally raised against the *Torpedo* electroplaque electric organ nAChR.²² These authors described a distribution of immunoreactivity that was frequently postsynaptic in rat cerebral cortex. The receptor subunit selectivity of this antibody has yet to be determined. Another antibody, mAb 270, which is known to recognize $\beta 2$ nAChR subunits, has provided low resolution film autoradiographs²³ and light microscopic images²⁴ in rat brain. However, thus far it has not proved compatible with tissue preparation procedures required for electron microscopic examination. Another group has recently reported immunocytochemical mapping of rat brain using polyclonal antibodies raised against fusion proteins corresponding to the $\beta 2$ subunit; little evidence was obtained for synaptically located nAChRs.²⁵ In contrast, junctionally located immunoreactivity has been reported in material stained using mAb 299, which is selective for $\alpha 4$ nAChR subunits.²⁶

At the light microscopic level, cholinergic projections and nAChRs are both widely dispersed throughout the neuroaxis. The pervasiveness of cholinergic systems has become particularly apparent with the development of sensitive anti-ChAT antibodies and immunocytochemical visualization methods,²⁷ so that previous reports of brain areas that possess nAChRs but lack cholinergic innervation must now be treated with caution.²⁸ In this respect, detailed ultrastructural information would be invaluable. However, very little is known at the ultrastructural level about the location of nAChRs with respect to cholinergic innervation. The early studies of Hunt, Arimatsu, Lentz, and their associates predated the development of a specific marker for cholinergic terminals, and provide no indication of the neurotransmitter phenotype(s) involved. Double-labeling studies in the CNS should by now be possible, but appear not to have been attempted.

Thus far, I have accepted the common assumption that in order to play a role in nicotinic cholinergic transmission, nAChRs must be preferentially located at syn-

apses. However, even where cholinergic transmission is synaptic in nature rather than paracrine, the critical question is really whether a *sufficient* number of nAChRs are present at the synapse to mediate the actions of released transmitter. How much is sufficient? In general, we do not know. This issue takes on more than academic interest when it is realized that some nAChRs may not be accurately located over the cell membrane. Although the evidence on this point is only suggestive, there are a number of neuronal systems where nAChRs appear to exist both at the somatodendritic level and on or near terminals. Examples include certain retinofugal pathways,²³ mesolimbic and nigrostriatal dopamine systems,²⁹ a number of thalamocortical projections,^{30,31} and the habenulointerpeduncular pathway.^{23,32} The prevalence of this phenomenon has led certain authors to refer to the possibility of "routing accidents."²³ In some neuronal systems, the receptors may indeed mediate cholinergic transmission at both levels of the neuron, but this is far from proven. For example, in the neostriatum, ultrastructural visualization has revealed few signs of cholinergic (or other) axoaxonic contacts.³³⁻³⁵ These findings indicate that presynaptic cholinergic modulation of dopamine release is unlikely to be important physiologically, unless it occurs at some distance to ACh release sites, despite the existence of presynaptic nAChRs in this brain region.

FUNCTIONAL STUDIES RELEVANT TO NICOTINIC CHOLINERGIC TRANSMISSION

Anatomical receptor mapping studies using radioligands and antibodies have indicated that cholinergic projections and nAChRs are widely encountered in the brain. However, little attempt has as yet been made to marry the two in order to assess the prevalence of nicotinic cholinergic transmission. Similarly, functional studies have concentrated on the actions of exogenous nicotine and acetylcholine, revealing that many neurons are sensitive to nicotinic agonists. However, few efforts have been directed at identifying possible sites in the CNS where *endogenous* ACh might be released onto nAChRs. Indeed, reasonably strong evidence for nicotinic cholinergic transmission in the brain has been obtained only in thalamus, substantia nigra pars compacta, and nucleus ambiguus. This work is now briefly reviewed.

Most thalamic nuclei exhibit high levels of nAChRs and related mRNA,^{10,36} and within several thalamic nuclei, direct application of nicotinic agonists has been shown to increase neuronal firing rates.^{37,38} The thalamus receives a major cholinergic input from the pedunclopontine tegmental nucleus and adjacent laterodorsal tegmental nucleus of the brain stem,⁴ and electron microscopic evidence also exists for synaptic innervation by ChAT-positive terminals.³⁹ Electrical stimulation in the vicinity of the pedunclopontine tegmental nucleus has been shown to excite thalamic relay neurons in the dorsal lateral geniculate nucleus with short latency, and this excitation was blocked by direct application of the nicotinic antagonist hexamethonium, providing evidence for nicotinic cholinergic transmission.⁴⁰

In the rat substantia nigra pars compacta, dopamine (DA) cells express nAChRs, as indicated by *in situ* hybridization histochemistry,³⁶ receptor autoradiography,²⁹ and electrophysiological recording.⁴¹ Electron microscopic visualization of ChAT-like immunoreactivity indicates that the DA neurons receive a cholinergic innervation,⁴² and retrograde tracing studies have identified the ipsilateral pedunclopontine tegmental nucleus as a major source of cholinergic fibers.^{43,44} Infusion of the excitant kainic acid in the vicinity of this ACh cell body group resulted in a dose-related and prompt excitation of identified nigral DA cells.⁴⁴ This excitation was shown to be induced by ipsilateral but not by contralateral kainate infusion

(consistent with the hodological findings), and was prevented by systemic administration of the nicotinic antagonist mecamylamine. Although a polysynaptic input cannot be ruled out, it appears likely that a direct cholinergic link exists between cell bodies in the pedunculopontine tegmental nucleus and the dopamine cells of the nigra.

Recently, evidence has also been provided for a nicotinic cholinergic input to motoneurons of the rat nucleus ambiguus. This nucleus possesses high levels of nAChR-related mRNA and protein.^{23,36} In anesthetized rats, responses to local administration of ACh and to the nicotinic agonist DMPP were shown to be blocked by nicotinic antagonists.⁴⁵ In subsequent intracellular recordings, application of ACh resulted in inward currents. These drug responses were most likely direct, because they persisted in the presence of tetrodotoxin and high extracellular manganese which inhibit sodium-dependent spikes and calcium-dependent stimulus-secretion coupling, respectively. Zhang *et al.* subsequently used retrograde tracing to identify a probable cholinergic input from cell bodies located in the rostral medulla, and the existence of nicotinic cholinergic transmission then received strong support in experiments in which electrical stimulation combined with direct antagonist application was performed *in vitro*.⁴⁶

IN VIVO REGULATION OF CNS nAChRs BY TREATMENT WITH ACETYLCHOLINESTERASE INHIBITORS

Another approach to assessing whether nAChRs are cholinergically innervated is to examine the effects of chronic *in vivo* administration of acetylcholinesterase inhibitors (AChEIs) on nAChR density in the brain. This kind of pharmacological treatment tends to increase cholinergic tone, and has been shown to decrease the density of brain muscarinic receptors.⁴⁷ In the first such experiments, high-affinity nicotinic agonist binding sites were also found to be depleted,^{47,48} suggesting that the corresponding nAChRs were cholinergically innervated. Subsequently, however, chronic treatment with AChEIs was found to *up-regulate* these sites.^{49,50} In another study, [¹²⁵I]- α -bungarotoxin binding sites were unaltered by chronic AChEI treatment despite concomitant decreases in [³H]nicotine binding.⁵¹

The use of AChEIs for defining possible sites of nicotinic cholinergic transmission suffers from a number of problems. Not only are the results discordant, but there are additional interpretational issues that have only recently surfaced. The first relates to possible neurotoxic effects of esterase inhibition⁵² that could conceivably lead to an irreversible loss of cells expressing nAChRs. In this context, it should be noted that the reversibility of AChEI-induced nAChR changes has yet to be examined.

The second issue relates to direct actions that certain AChEIs exert on CNS nAChRs. Albuquerque and colleagues have amassed considerable evidence that in rat brain the AChEI physostigmine can produce activation via a direct action on nAChRs which is mediated by a site on α -subunits that is distinct from the ACh binding domain;^{53,54} at higher concentrations, an antagonist action was seen.⁵⁵ It should be noted that the receptors under study in these experiments were of a subtype in which responses to classical nicotinic agonists were blocked by α -bungarotoxin.

In our own experiments, we have studied the effects of several AChEIs on a nicotinic response that is mediated by receptors *insensitive* to α -bungarotoxin. The response in question is [³H]dopamine release from superfused rat striatal synaptosomes, which can be evoked by nicotine and other agonists in a concentration- and calcium-dependent manner.⁵⁶ Physostigmine, neostigmine, tacrine, and diisopropyl-

fluorophosphate (DFP) all reduced nicotine-induced DA release in a concentration-dependent manner (Fig. 1), but physostigmine and tacrine were clearly more potent than DFP.⁵⁷ Tests of pharmacological selectivity involved comparisons between nicotine and other secretagogues (other nicotinic agonists, amphetamine, and high K^+) (Fig. 2). These tests revealed that physostigmine blocked nicotinic responses in a selective fashion, indicating a probable action at nAChRs. Tacrine, in contrast, acted nonselectively, and may have exerted its actions entirely independently of

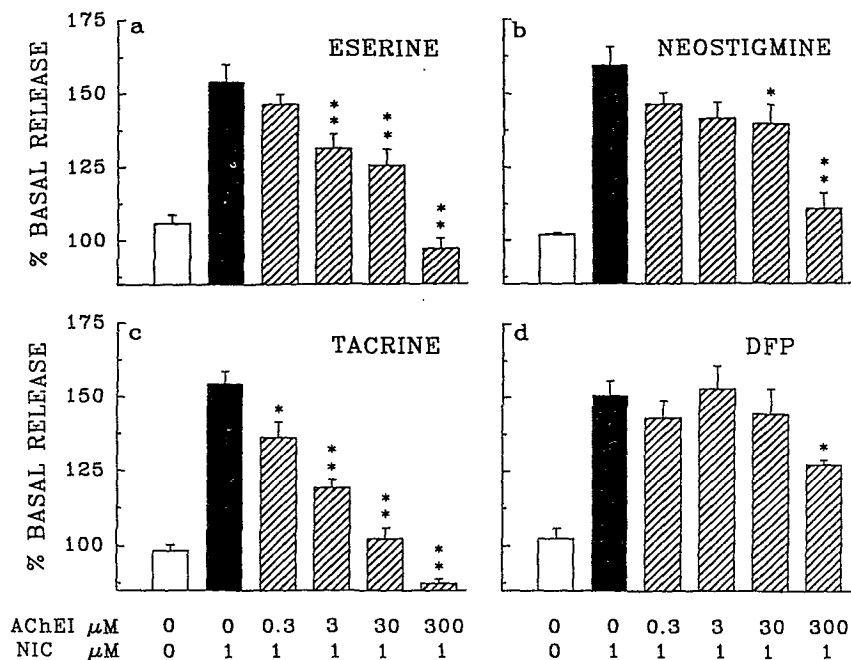


FIGURE 1. Effects of eserine (a), neostigmine (b), tacrine (c), and DFP (d) on $[^3H]$ dopamine release induced by nicotine from striatal synaptosomes. Synaptosomes were superfused with buffer in the presence or absence of AChE inhibitor (0.3–300 μM) for 35 min prior to challenge with nicotine 1 μM or buffer. The vertical axis shows mean \pm SE mean peak release expressed as a percentage of basal release. Superfusion channels per condition: $n = 10$ –20 (a), 8–11 (b), 7–10 (c), 7–14 (d). * $p < 0.05$, ** $p < 0.01$ vs. nicotine alone (Dunnett's test). (Clarke *et al.*⁵⁷ Reproduced, with permission, from the *British Journal of Pharmacology*.)

nAChRs. Antagonism by physostigmine was not preceded by a nicotine-like stimulation, suggesting that it was not the result of agonist-induced desensitization. We also considered the possibility that the observed blockade resulted from inhibition of AChE, which might conceivably have produced sufficiently elevated ACh levels in the perfusate to lead to nAChR desensitization. However, a clear dissociation was found between nicotinic block and esterase inhibition, supporting the conclusion that physostigmine directly blocks the nAChRs under study. The same concentra-

tions of physostigmine that produced nicotinic antagonism are commonly used *in vitro* in order to inhibit AChE; whether *in vivo* physostigmine treatment would significantly affect nAChR function is not clear.

The third issue that complicates the interpretation of nAChR regulation experiments relates to uncertainties concerning the mechanisms by which receptor changes

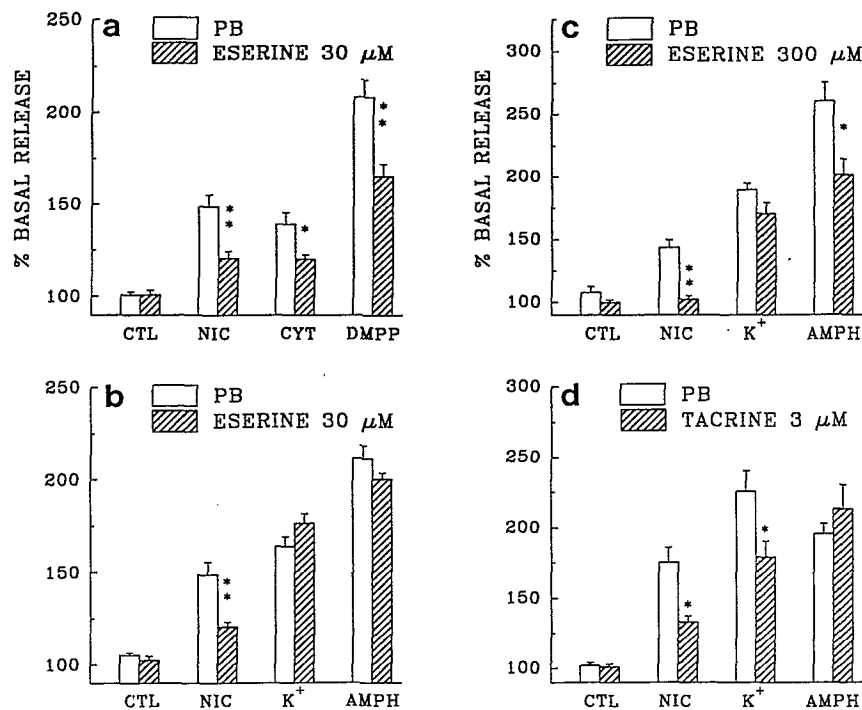


FIGURE 2. Pharmacological selectivity of blockade by eserine or tacrine. In all assays, synaptosomes were superfused for 35 min with or without AChE inhibitor (cross-hatched and open bars, respectively), prior to acute challenge with [³H]dopamine secretagogue. (a) Challenge with nicotine 1 μ M, cytosine 10 μ M, DMPP 10 μ M or buffer alone, with or without eserine (30 μ M). (b) Challenge with nicotine 1 μ M, K⁺ 12 mM, (+)-amphetamine 0.3 μ M or buffer alone, with or without eserine 30 μ M. (c) As for (b), except eserine 300 μ M was used. (d) As for (b), except tacrine 3 μ M was used. The vertical axis shows mean \pm SE mean peak release expressed as a percentage of basal release. Superfusion channels per condition: $n = 8-12$ (a), 13-17 (b), 5-8 (c), 6-13 (d). * $p < 0.05$, ** $p < 0.01$ vs. AChEI-free condition at same agonist concentration (Student's t test with Bonferroni adjustment). (Clarke *et al.*⁵⁷ Reproduced, with permission, from the *British Journal of Pharmacology*.)

are triggered. Paradoxically, chronic *in vivo* treatment with nicotine or other centrally acting agonists typically *up-regulates* high-affinity [³H]agonist binding site density.⁵⁸ It has been suggested that this occurs because in the doses administered, nicotine may act as a "time averaged" antagonist.^{59,60} However, this plausible notion seems to be put in some doubt by our recent observations that treatment with the quasi-

irreversible CNS nicotinic antagonist chlorisondamine did not significantly alter [^3H]nicotine binding site density, and did not prevent the up-regulation resulting from chronic nicotine treatment, despite demonstrated CNS nicotinic blockade.⁶¹

CONSEQUENCES OF CNS NICOTINIC BLOCK

If nicotinic cholinergic transmission is important to CNS functioning, the consequences of CNS nAChR blockade should be serious. Many studies have examined the effects of centrally active nicotinic antagonists on neurochemical and behavioral effects in animals, but the interpretation of these studies is complicated by several issues. First, almost all investigators have relied on the antagonist mecamylamine. It is not clear how selective this drug is for nAChRs over NMDA-type glutamate receptors.⁶² In addition, because mecamylamine has been found to act in an insurmountable fashion in the CNS,⁶² doses of this drug that completely block the effects of administered nicotine should also be sufficient to block the actions of endogenous ACh. However, where mecamylamine has been found to be active in behavioral tests, this has often only occurred at high doses.

A second problem lies in the frequent use of hexamethonium as a control for the peripheral effects of mecamylamine. Although both nicotinic antagonists are ganglion blockers, there appears to be little information available on relative potency and duration of action in the rat. Thus, the commonly performed comparison of mecamylamine 1 mg/kg s.c. versus hexamethonium 5 mg/kg s.c. is based more upon tradition than on hard data.

The possible existence of nicotinic autoreceptors poses further problems of interpretation. Thus, in certain isolated tissues, ACh release is enhanced by a direct action of nicotinic agonists.^{63,64} It is conceivable, therefore, that nicotinic antagonists, by blocking autoreceptors, may inhibit cholinergic transmission *in vivo* at any synapses where *postsynaptic* cholinergic receptors are muscarinic rather than nicotinic.

Although most investigators have used mecamylamine as the nicotinic antagonist of choice, the bisquaternary compound chlorisondamine provides an alternative method with which to investigate the effects of central nAChR blockade. Central blockade can be achieved by administering chlorisondamine either in a high subcutaneous dose or in a much lower dose given intracerebroventricularly.⁶⁵ In contrast to the persistent central block, which lasts for many weeks after a single administration, ganglionic blockade is only transient.⁶² Where studied, chlorisondamine has been found to antagonize the effects of nicotine in an insurmountable fashion.^{56,66} Thus far, it does not appear that the chronic nicotinic blockade that follows chlorisondamine administration results in any major functional impairment.^{65,67,68} This might imply that nicotinic cholinergic transmission in the CNS is not critical to important psychobiological processes, but may also reflect the capacity of the nervous system to adapt.

CONCLUSION

Studying the actions of nicotine should in principle tell us something about cholinergic neurotransmission in the brain. However, the relationship appears to be complicated, and it is not at all clear how many CNS nAChRs are really innervated by ACh. Although cholinergic fibers and nAChRs have been mapped in some detail, few attempts have been made to demonstrate transmission. Indeed, some observa-

tions suggest that nAChR localization may *not* be precisely controlled. Another way to identify possible sites of cholinergic transmission is to examine the *in vivo* regulation of nAChRs by cholinesterase inhibitors. However, recent evidence suggests that functional status may *not* be an important factor in the regulation of CNS nAChRs.

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